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INSTITUTE OF MEDICINE

Roundtable for the Development of Drugs
and Vaccines Against AIDS

Progress Report November 1992

The Roundtable for the Development of Drugs and Vaccines Against AIDS, established in December 1988, is composed of leaders from government, the pharmaceutical industry, academia, and patient advocacy. Its mission is to explore impediments to the rapid availability of safe, effective drugs and vaccines for HIV infection and AIDS. The Roundtable meets quarterly and holds workshops and conferences on topics selected for their urgency and for their potential to expedite drug and vaccine development and availability, if addressed constructively. After completing its first term in December 1991, the Roundtable was renewed for another two-year term. New co-chairs and seven additional members have been appointed to serve on the Roundtable (see attached roster in Appendix A).

During their approximately four-year tenure, Roundtable members have reviewed aspects of the state of scientific knowledge relevant to the design of therapeutic and preventive agents; examined some of the impediments to successful HIV drug and vaccine development, approval, and availability; and discussed strategies to promote the development of drugs and vaccines against AIDS. As a result, the Roundtable has drawn considerable attention as a forum for exploring the scientific basis of innovative policies being tested in the AIDS arena and for helping to foster increased communication among public- and private-sector representatives interested in furthering the development of effective HIV drugs and vaccines. As described below, the Roundtable's activities comprise a collection of discrete themes that are unified by their having been identified as important obstacles to successful HIV drug and vaccine development.

The Potential Value of Research Consortia in the Development of Drugs and Vaccines Against HIV Infection and AIDS

The Roundtable's first task was to respond to a request from the Public Health Service (PHS). The AIDS Amendments of 1988 directed the PHS to request a study of whether research consortia might enhance HIV drug and vaccine development and, if so, what measures the government should take to encourage their formation. The Roundtable report, *The Potential Value of Research Consortia in the Development of Drugs and Vaccines Against HIV Infection and AIDS* (September 1989), suggested that although a massive consortium approach to HIV research was not warranted, there are two areas of research that might profit from such an approach: drugs for opportunistic infections (OI) and development of animal models.

Surrogate Endpoints in Evaluating the Effectiveness of Drugs Against HIV Infection and AIDS

In September 1989, the Roundtable sponsored a public conference to examine the use of surrogate endpoints in evaluating AIDS drugs for effectiveness and approving them for marketing. Surrogate endpoints are laboratory markers that might be substituted for "true" endpoints of interest, such as increased survival or the occurrence of opportunistic infections, in order to shorten the time necessary to evaluate new drugs.

Investigators presented data on potential surrogate endpoints for drug approval, including CD4+ T lymphocytes, p24 antigen, plasma viremia, and some clinical measures. The risks and benefits of approving drugs on the basis of surrogate measures of effectiveness were also discussed. A summary of the conference findings, *Surrogate Endpoints in Evaluating the Effectiveness of Drugs Against HIV Infection and AIDS*, was published in June 1990.

HIV Vaccine Development

The Roundtable addressed HIV vaccine development questions in a two-stage process. In January 1990, Roundtable members and invited guests explored the scientific challenges and obstacles to development of a vaccine for HIV, including vaccines to generate cell-mediated immunity and protective antibodies, and vectors and genetically engineered vaccines. They also reviewed progress in animal model experimentation and experiments to induce post-infection immunity using inactivated whole virus in HIV-infected human volunteers. In September 1990, a larger invitational workshop shifted the focus to policy issues related to the science of vaccine development. Workshop participants and Roundtable members examined the determinants of pharmaceutical companies' decisions to enter the field of HIV vaccine research (including questions of liability and their impact on industry decision making), enhancement of government-industry collaboration, criteria for moving from animal to human experimentation, planning large-scale efficacy trials, the potential clinical applications of an HIV vaccine, and mechanisms to promote distribution of an effective HIV vaccine. A summary of the January 1990 workshop was distributed to Roundtable members and sponsors in June 1990.

Expanding Access to Investigational Therapies for HIV Infection and AIDS

Several mechanisms have been created to provide increased access to investigational therapies for persons with life-threatening disease; two examples of such mechanisms are the treatment investigational new drug (IND) and parallel track program. In March 1990, the Roundtable held a conference to consider these and other current policy options to improve patient access to new therapies. Participants reviewed the role of treatment IND regulations, issues of cost and coverage for expanded access programs, the potential for conducting research through programs for distribution of unapproved drugs, and the role of expanded access programs in reaching the medically disenfranchised. The conference report, *Expanding Access to Investigational Therapies for HIV Infection and AIDS*, was issued in March 1991.

Drug Development for Pediatric HIV Infection and AIDS

In June 1990, the Roundtable convened a small workshop on developing drugs for infants and children with HIV infection. Speakers and Roundtable members discussed a number of topics, including the epidemiology of HIV infection among childbearing women and newborns; vertical transmission; reliable HIV diagnostic assays; prognostic markers, natural history, and staging of infection in children; and social issues pertaining to HIV-infected children. The workshop concluded with a review of the development and approval process for drugs to treat pediatric HIV infection and AIDS.

Immune Reconstitution Therapies for People with AIDS

The Roundtable turned its attention to immune reconstitution as a potential life-saving therapeutic intervention for people with late-stage AIDS in December 1990, when a small group of scientists convened to discuss the development of a research agenda in this relatively young area. Investigators provided brief overviews of human T cell development, the role of cytokines in the disease process and their potential therapeutic applications, bone marrow transplantation, thymic transplantation, and adoptive immunotherapy. The one-day workshop was structured to allow extensive discussion of the formulation of a research agenda in this area.

AIDS-Related Opportunistic Infections

The paucity of drugs to treat opportunistic infections is a matter that has concerned the Roundtable since its inception and was briefly considered in the 1989 report on research consortia. In April 1991, a conference was held to review what is known about the pathogenesis and treatment of the major AIDS-related opportunistic infections and to explore obstacles to the development of effective OI drugs. Conference participants also addressed data collection and FDA regulatory requirements for the expeditious approval of OI drugs, as well as the use of community-based trial networks in gathering information on therapeutic effectiveness more quickly. A synopsis of this conference was published in April 1992.

Understanding HIV Pathogenesis

In July 1991, the Roundtable sponsored an invitational workshop to examine the current understanding of HIV pathogenesis and the scientific challenges that remain in further elucidating the pathogenic mechanisms of HIV and in developing effective drugs and vaccines. Workshop participants explored a variety of topics related to HIV pathogenesis, including: what is known about the accessory genes of HIV, their specific functions, and their role in pathogenesis; biological variability of HIV and determinants of viral tropism; the nature of the immune deficit associated with HIV infection and role of immune activation in accelerating disease development; whether the HIV-associated immune deficit is potentially reversible; the immune response to HIV (e.g., viral targets for cellular and humoral immune response and whether there is evidence of immune escape by the virus); speculation on what might constitute protective immunity; and targeted drug design and the identification of novel targets for antiviral therapeutic intervention. A summary of the workshop discussions is attached (see Appendix B).

Investigational Therapies and Off-Label Use of Approved Drugs

The Roundtable concluded its third year, in December 1991, with an invitational workshop on investigational therapies and off-label use of approved drugs to treat HIV infection and cancer. During the workshop, participants discussed the extent of off-label uses of prescription drugs, the current FDA position on off-label use of approved therapies, early dissemination of clinically important treatment information to health care providers (and the pharmaceutical industry's participation in such dissemination activities), the development of appropriate reimbursement criteria for investigational treatments and off-label indications, and ways to expedite the process for second-line approval of drugs. A synopsis of this workshop will be distributed shortly.

Combination HIV Therapies

The use of combination antiretroviral therapies to treat HIV infection and AIDS is becoming increasingly common, particularly as a means of counteracting the development of HIV drug resistance. In September 1992, the Roundtable convened an invitational workshop to review the current knowledge of HIV drug resistance and clinical experience with combination HIV therapies. Roundtable members and workshop participants examined a variety of topics, including the implications of viral resistance for the development of effective antiretroviral therapy; current experience with combination antiretroviral and immune-based therapies; combination therapy study design, including populations for inclusion in such studies and trial endpoints; and perspectives on fostering productive collaboration among multiple drug companies, NIH, FDA, and university investigators and on facilitating expeditious combination HIV drug development and approval. A summary of the workshop proceedings is expected in early 1993.

Future Plans

Continuing debate has surrounded the development and evaluation of preventive HIV vaccines. In light of the scientific and policy questions that remain in this area, the Roundtable decided to explore these important concerns in its December 1992 workshop. Among the issues that will be addressed are: the current status of HIV vaccine development efforts; guidelines for decision making in launching vaccine efficacy trials; designing vaccine trials and establishing realistic expectations for trial outcome; community perspectives on preventive HIV vaccine trials; behavioral and ethical considerations in planning and conducting vaccine trials; public health implications of an effective HIV vaccine; liability concerns and options for resolution; economic considerations in vaccine research and development; and likely utilization and distribution of preventive HIV vaccines.

Plans for the Roundtable's 1993 workshops are in progress. The April workshop is expected to focus on horizons in therapy for HIV infection and AIDS. This workshop will be a follow-up to the 1990 meeting on immune reconstitution therapy and will examine novel and, perhaps radical, approaches to treating HIV disease. The September workshop is scheduled to explore the changing demography of the HIV epidemic and its impact on clinical research. The topic for the final workshop in December has not been identified as yet.

APPENDIX A

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APPENDIX B

UNDERSTANDING HIV PATHOGENESIS: IMPLICATIONS FOR DRUG AND VACCINE DEVELOPMENT

Summary of a Workshop
July 26, 1991

Institute of Medicine
Roundtable for the Development of
Drugs and Vaccines Against AIDS

Since the human immunodeficiency virus (HIV-1) was first isolated and identified as the etiologic agent of acquired immune deficiency syndrome (AIDS), biomedical researchers have made substantial gains in defining important aspects of the genetic structure and *in vitro* biology of the virus. Yet progress in elucidating the natural history and pathogenesis of HIV infection has proceeded more slowly. Emerging scientific data are now illuminating a preliminary, but increasingly, coherent framework for understanding the pathogenesis of HIV-associated disease. A more complete description of the pathogenesis and natural history of HIV infection is likely to facilitate the development and application of improved therapeutic interventions.

VIRAL PATHOGENESIS OF HIV INFECTION AND AIDS: CURRENT UNDERSTANDING AND UNRESOLVED QUESTIONS[1]

To understand more fully the pathogenesis of HIV infection, researchers will need to define the level of viral burden and expression that are present at various stages of disease. They will also need to describe further the various factors that regulate the level of viral replication. The prediction, based on early studies, that few target cells in HIV-infected individuals actually harbor and express the virus has remained a fundamental difficulty in understanding the pathogenesis of infection. The recent application of more sensitive methods of viral detection, however, indicates that both the viral burden and magnitude of expression may be substantially higher than previously believed. The level of replication *in vivo* appears to represent a balance between the complex viral mechanisms that regulate HIV gene expression and the ability of the host immune system to recognize and eliminate infected cells. Activation and attenuation of HIV production also appear to depend on important cellular factors involved in the normal host response to immunologic or inflammatory stimuli.

Studies of the HIV-1 life cycle have demonstrated that the character of viral gene expression differs at various points following viral infection. The expression of HIV-1 genes assumes two essential stages - early and late - that are distinguished by the pattern of viral ribonucleic acids (RNA) present in infected cells. In the "early" stage, shortly following infection, the predominant viral RNA transcripts are the multiply-spliced transcripts that encode the viral *tat*, *rev*, and *nef* gene products. It is thought that this "early" pattern derives from the paucity of HIV *rev* protein present shortly after infection. The *rev* protein plays an essential role in viral replication and regulates the pattern of viral RNA transcripts that are synthesized. The *rev* gene acts, in an incompletely defined manner, to regulate either the splicing or nuclear to cytoplasmic transport of specific viral RNA pieces. In the absence of *rev*, only the multiply-spliced transcripts that encode the viral regulatory gene products are available for translation in the cytoplasm. As time passes following infection and as *rev* protein accumulates, the "late" stage of gene expression occurs during which the unspliced or singly-spliced viral RNAs are expressed. These transcripts encode the virion structural and enzymatic constituents, and their expression allows viral assembly and subsequent transmission to proceed. Additionally, recent evidence suggests that the complex viral regulatory process, which underlies transition from the "early" to "late" stages of the HIV-1 life cycle, may also play an important role in the virus's ability to assume an attenuated or latent state of infection.

Studies of HIV-infected individuals indicate that far more CD4+ lymphocytes may be infected in the peripheral blood than those that actually express detectable levels of viral RNA or protein. Experiments in tissue culture models of HIV latency suggest a possible explanation for this observation, which derives from an intricate interplay between viral and cellular regulatory factors. HIV transcription and production are activated by antigenic, mitogenic, or inflammatory stimuli. This transcriptional activation is mediated in large part by the cellular transcription factor referred to as NF-jB. This factor serves an important function in activating the expression of a number of cellular genes that are essential to

[1]This section is based on material presented by David Baltimore.

generate an immune response. In an inactivated state, a cell infected with HIV may contain little active NF-jB and, thus, HIV transcription is limited. When the level of HIV transcription is low, only a small amount of *rev* protein is produced. As a result, the pattern of viral transcripts resembles the "early" phase of gene expression - that is, the stage during which the multiply-spliced messenger RNAs that encode the regulatory gene products are preferentially produced and those that encode the virion constituents are rare. With immunologic or inflammatory stimulation, the NF-jB is activated, resulting in increased HIV transcription. As HIV transcription accelerates, the level of *rev* protein that is synthesized also increases. HIV-1 gene products that are characteristic of the "late" stage of the viral life cycle can then be synthesized, and viral assembly can proceed. By remaining in the "early" stage of its life cycle in quiescent cells, the virus synthesizes little of the immunogenic structural proteins that target an infected cell for immunologic elimination. Further, because expression of the HIV envelope glycoprotein (a "late" gene product) can result in direct cytopathic consequences for an infected cell, carefully regulated control of its expression may be essential. Both of these factors may serve to facilitate viral persistence in an infected host.

UNDERSTANDING THE VIRAL PATHOGENESIS OF HIV INFECTION AND AIDS[2]

Definition of the range of cells that are targets for HIV infection is essential to elucidate the process of viral pathogenesis. The CD4+ T lymphocyte (or so-called, helper-inducer T cell) is known to be the prime target for HIV infection in the peripheral bloodstream, while macrophages are important targets for viral infection in the peripheral tissues, the central nervous system, and perhaps, the lymphoid organs. Recent attention has also focused on the possibility that follicular dendritic cells play an important role as early targets of HIV infection, although this is still controversial.

The CD4 cell surface molecule that phenotypically defines the helper-inducer population of lymphocytes, which is profoundly depleted in persons with AIDS, is, in fact, the receptor that HIV uses to gain entry into its target cells. The CD4 molecule serves as the viral receptor on both lymphocytes and macrophages. The process of HIV infection is initiated when the virus particle binds to CD4 present on the surface of a cell via the viral envelope glycoprotein, gp120. The virus then gains entrance to the cell through a process of membrane fusion mediated by the HIV envelope protein, gp41, which is physically associated with gp120. (Both proteins are encoded by a single gene whose translation product, gp160, undergoes proteolytic processing to the two constituent parts - gp120 and gp41).

Although lymphocytes and macrophages can both serve as targets for HIV infection, by virtue of their expression of the CD4 molecule, certain isolates of HIV will preferentially infect one or the other of these cell types. The genetic basis of the preferential tropism of HIV isolates for specific target cells has recently been mapped to a region of gp120, known as the V3 loop. This region also represents the principal neutralization domain that is recognized by antiviral antibodies, which block HIV infection *in vitro*. Although the envelope glycoprotein has been clearly implicated in effecting viral tropism, the mechanism by which this occurs has not yet been described.

With the identification of CD4 as the HIV receptor, biomedical researchers proposed an imaginative approach to antiviral therapy, whereby soluble versions of CD4 were produced to competitively block the process of virus binding to potential targets. In fact, this approach worked well in tissue culture models that utilized standard laboratory isolates of HIV and immortalized T cell lines. However, soluble CD4 was without demonstrable antiviral effect *in vivo*. The apparent explanation for this discrepancy is

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[2]This section is based on material presented by Robert Gallo.

that primary isolates of HIV, as opposed to those adapted for growth *in vitro*, are poorly inhibited by soluble CD4. This experience highlights the difficulties in extrapolating certain aspects of *in vitro* studies to the complexities of HIV biology *in vivo*.

In addition to mediating HIV binding and entry into target cells, the gp120/gp41 envelope glycoprotein complex also plays a major role in the cytopathic consequences of HIV infection. Expression of the gp120/gp41 complex in a CD4+ T cell can result in a process of membrane fusion between neighboring cells, which is termed syncytia formation. Although this is an obvious result of HIV infection *in vitro*, its role in the process of CD4+ T cell depletion *in vivo* is not known. Other mechanisms for T cell depletion have been proposed: the possible influence of the HIV *nef* gene on T cell growth and the inappropriate targeting of uninfected CD4+ lymphocytes for immune clearance through an "innocent bystander" mechanism when lymphocytes bind free gp120 to their cell surface. An essential aspect of future investigations should be an improved understanding of the respective contributions of the direct and indirect mechanisms of T cell depletion that follow HIV infection.

The factors that underlie the loss of CD4+ lymphocytes, following HIV infection, are not clearly understood nor are the factors that contribute to the immune system's failure to replace these lost cells. This may be due, in part, to a fundamental limitation of T cell repopulation in adult humans, although little is known about this important process. Alternatively, HIV infection may result in direct damage to the thymus where T cell differentiation and production occur. A critical goal for future studies is to define the kinetics of the loss and replenishment of T cells following HIV infection.

Further information is needed regarding the important effects of HIV-associated disease that occur outside the T lymphocyte population. Such manifestations include the neurological sequelae of HIV infection, as well as two types of malignancies - B cell lymphoma and Kaposi's sarcoma (KS) - often seen among persons with AIDS. Aggressive B cell lymphoma is emerging as a prominent and increasingly frequent AIDS diagnosis, although its etiology is unknown. Several possible etiologies, however, have been offered. B cell lymphomas may be the result of an as yet unidentified infectious agent or the outcome of polyclonal B cell activation that occurs in HIV-infected persons. Or, these lymphomas may arise due to the failure of immune surveillance against malignant transformation. Kaposi's sarcoma, the other characteristic HIV-associated malignancy, is also poorly understood. The role of HIV in the etiology of KS, acting in either a direct or indirect role, is not well defined but remains an active area of research.

ACCESSORY GENES OF HIV[3]

Three structural and enzymatic gene products - *gag*, *pol*, and *env* - are common to all retroviruses. In addition to these gene products, lentiviruses (such as HIV-1 and HIV-2) encode an array of other products. These auxiliary genes are termed: *tat*, *rev*, *nef*, *vif*, *vpr*, *vpu*, and *vpx*. Of these genes, *tat*, *rev*, *vif*, and *nef* are present in all lentiviruses. *Vpu* is present in HIV-1 but absent from all other members of the lentivirus subfamily. *Vpx*, on the other hand, is present in all of the immunosuppressive lentiviruses, except HIV-1 and the simian immunodeficiency virus (SIV) isolate from mandrills. Based on gene sequence comparisons, scientists have postulated that *vpx* is a duplicate *vpr* gene, and that the presence of *vpr* in HIV-1 and SIV from mandrills may compensate for the absence of *vpx*.

The *tat* and *rev* gene products are essential for HIV replication. *Tat* is required to efficiently synthesize viral RNA and to facilitate elongation of HIV RNA transcripts. *Rev*, as previously discussed, controls the pattern of spliced HIV RNA transcripts that are expressed in infected cells. This process

[3]This section is based on material presented by Ronald Desrosiers.

occurs either through regulation of the splicing of viral RNA transcripts or through selective control of viral RNA transport from the nucleus. Because both of these gene products are essential for HIV replication, they provide promising targets for future development of antiviral agents.

Scientists have had difficulty in determining the function of the remaining "non-essential" gene (i.e., *nef*, *vpr*, *vpx*, *vpu*, *vif*) products, in part because they are not critical to viral replication. Furthermore, no assays have been identified, to date, for measuring the function of these gene products *in vitro*. In the absence of reliable, reproducible assays, various studies of their function have produced conflicting results. Although these gene products are not essential for viral replication in tissue culture, their conservation among the diverse members of the lentivirus subfamily of retroviruses suggests that these gene products play important roles in the *in vivo* processes of infection and pathogenesis.

Convincing evidence for this view comes from recent analyses of the *nef* gene. As previously noted, *nef* is dispensable for viral replication *in vitro*. Scientists have reported that *nef* binds GTP, acts as a GTPase, and undergoes autophosphorylation, but these findings have been disputed. Scientists have also reported that *nef* has a negative, positive, or no effect on viral transcription. The acronym, nef, derives from earlier suggestions that it served as a *Negative Factor*. Recent studies in the SIV model, however, have indicated that although mutation of the *nef* gene has no appreciable effect on SIV replication *in vitro*, it destroys the ability to cause disease in the rhesus macaque. *Nef* plays an as yet unidentified function required for the maintenance of a high virus load and for disease development in infected animals. Determination of the *in vivo* function of *nef* remains a critical area for future investigation.

The *vif* (Virion Infectivity Factor) gene is present in all primate lentiviruses. Mutation of *vif* substantially decreases but does not abolish viral replication in tissue culture. The magnitude of this effect, however, depends on the target cells that are used. *Vif* seems to be particularly important for cell-free virus transmission. Preliminary studies have shown that *vif* may be associated with the Golgi apparatus and may affect the processing of the viral gp41 envelope glycoprotein.

The *vpr* gene is present in all primate lentivirus groups, except SIV_{agm}. The *vpr* protein is found in detectable quantities in virions, although its function is unknown. Mutation of *vpr*, however, has no apparent effect on viral replication. Some scientists believe that it may play a role in viral infection of macrophages.

The *vpx* gene is found in HIV-2 and SIV_{mac} virus groups. The *vpx* protein is associated with the virion, perhaps in high concentrations. As previously discussed, however, this gene is dispensable for virus replication in tissue culture and may have originated via duplication of the *vpr* gene. It is hoped that future studies will clarify the function of *vpx*.

The *vpu* gene is present only in the HIV-1 group. The *vpu* protein has been reported to increase the export of virus from infected cells, perhaps by influencing the formation of intracellular complexes between gp120 and CD4 molecules.

As the preceding discussion indicates, the function of the "non-essential" gene products of the immunosuppressive lentiviruses is unclear. A more complete description of their function is essential to future progress in understanding viral transmission, disease pathogenesis, and in developing antiviral drugs that are specifically designed to target their function.

BIOLOGICAL VARIABILITY OF HIV[4]

The mutation rate of retroviruses, including HIV-1, over a single replication cycle is quite high - estimated to be between 10^{-3} to 10^{-5} per nucleotide per round of replication. When this rate is extrapolated to project the mutation rate that occurs *in vivo*, it is estimated that the rate of change is 0.1 to 1 percent per year. The high mutation rate of retroviruses stems from the lack of a "proof-reading" function in the viral reverse transcriptase, such that polymerase errors cannot be corrected. The biological significance of HIV-1 sequence variation is not well understood but is likely to be important in understanding the basis of viral persistence and pathogenesis.

A confounding feature in the experimental design of early studies of HIV-1 genetic variability was the reliance on *in vitro* propagation of viral isolates prior to genetic or biologic characterization. Only later was it discovered that the process of culturing HIV *in vitro* rapidly selects for viral variants, which may not accurately represent the population of viruses present in the original inoculum. Thus, the isolates that were typically studied frequently did not reflect the distribution or character of viral variants present in an HIV-infected individual. The subsequent development of the polymerase chain reaction (PCR) technique has permitted the direct cloning and sequence analysis of viruses as they exist *in vivo*.

A fundamental question concerning the nature of sequence variation *in vivo* is whether the process is random, or whether it reflects the influence of selective pressure exerted by the host immune system. Resolution of this question is important for future understanding of the immunopathogenesis of infection and the mechanism of HIV persistence *in vivo*, as well as for vaccine development efforts.

In the few instances in which primary HIV infection has been studied in detail, it appears that viral sequence variation can occur quite soon after establishment of infection. The evolution of viral populations in infected individuals is the focus of a number of ongoing descriptive analyses. Viral variation is thought to play an important role in the virus's escape from immune surveillance, although this is not yet clear. Biological variation may also be of pathogenic significance, particularly if early suggestions concerning the possible acceleration of immunodeficiency coincident with the appearance of more highly replicative viruses prove correct. Sequence variation is known to play a role in determining the cellular tropism of a given isolate. In this instance, sequences within the V3 loop of gp120 appear to be responsible for determining a variant's tropism for T cells or macrophages.

The pronounced sequence variability of HIV-1 also has important practical implications for drug and vaccine development efforts. Rapid sequence evolution of HIV plays a critical role in the development of drug resistance. In addition, candidate HIV vaccines will need to accommodate the range of sequence variation in their design, testing, and application.

IMMUNOPATHOGENESIS OF HIV INFECTION AND AIDS: CURRENT UNDERSTANDING AND UNRESOLVED QUESTIONS[5]

The profound, deleterious impact that HIV has on the human immune system may reflect the susceptibility of the essential population of CD4+ lymphocytes and macrophages to viral infection and killing. Lymphocytes and macrophages play essential functional and regulatory roles in the human

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[4]This section is based on material presented by Irvin Chen.

[5]This section is based on material presented by Max Cooper and Anthony Fauci.

immune system. The impairment of these important functions would be expected to have numerous and potentially deleterious effects. The varied pathology resulting from HIV infection validates this expectation.

Progressive loss of CD4+ lymphocytes following HIV infection indicates that the capacity for T cell replenishment is ultimately outstripped by the process of lymphocyte depletion. Currently, scientists' limited knowledge about the balance and control of lymphocyte production in adult humans presents a challenge in understanding the kinetics of CD4+ lymphocyte loss associated with HIV infection.

The progress of HIV infection almost certainly represents a balance between immunologic containment of viral replication and the control of viral gene expression. The details of this process are unclear, but important insights have recently emerged. Shortly following exposure to HIV, an acute viral syndrome, which represents primary HIV infection, occurs in some individuals. In the absence of preexisting antiviral immunity, HIV can initially replicate to high levels and become readily detectable in cell-free plasma and in a substantial proportion (up to one percent) of peripheral blood lymphocytes. During this period, there is a transient drop in the number of CD4+ lymphocytes, which may result from lymphocyte redistribution or loss. It is possible that much of the damage to the host immune system, which may presage future deterioration, occurs during this period of primary infection.

The high level of virus expression that occurs during primary infection declines with the appearance of antiviral antibodies. The development of antiviral cytotoxic immune responses is also likely to play a role in decreasing the extent of active viral replication, although little information on this important topic is currently available.

With the decline of virus replication following the acute viral (or seroconversion) syndrome, the chronic phase of infection begins. This period was previously considered the "latent" stage, but such a characterization represents some confusion of the definitions of clinical and microbiological latency. Recent research results have convincingly shown that HIV replication and expression continue at all stages of the disease process and, thus, HIV is never truly latent (from a microbiological perspective).

During the chronic phase of infection, the number of infected cells present in the peripheral blood and the magnitude of plasma viremia are both low. Within the peripheral bloodstream, CD4+ T cells constitute the predominant reservoir of HIV-infected cells. Monocytes in the peripheral blood apparently are not major target cells.

In asymptomatic HIV-infected individuals, an estimated one in 10,000 to one in 100,000 CD4+ T cells in the peripheral blood are infected with HIV-1, and only very low levels of plasma viremia can be detected. The subsequent increasing viral burden heralds the development of progressive immunologic impairment. The proportion of infected CD4+ lymphocytes frequently rises coincident with an accelerated decline in total number. During this period of evolving immunodeficiency and progression to clinical AIDS, viral expression increases with the recrudescence of significant plasma viremia, elevated p24 antigen levels, and increased viral RNA synthesis. An increasing viral burden can herald the development of clinical AIDS; it may also reflect the failure of the immune system to contain HIV infection.

Early investigations of viral burden and expression during various stages of HIV disease have relied primarily on studies involving peripheral blood cells. Recent work, however, shows that lymphoid tissues exhibit a high viral burden and increased levels of viral expression. The localization of viral replication in the lymphoid tissues may have significant implications for disease pathogenesis. As previously noted, immunologic or inflammatory stimuli [including the cytokines known as tumor necrosis factor (TNF), interleukin 6 (IL-6) and granulocyte macrophage colony stimulating factor (GM-CSF)] enhance HIV production in infected cells. These cytokines all activate expression of the cellular transcription factor, NF-jB, which in turn augments HIV gene expression.

Concentration of HIV-infected cells at the anatomic sites of the immune response may also enhance viral expression. T cells involved in the generation of an immune response to a given antigen may thus bear the brunt of the cytopathic consequences of HIV-1 infection. In addition, recent evidence indicates that viral infection of the thymus itself may be important in understanding the failure of the immune system to compensate for T cell loss due to HIV infection.

IMMUNE DEFICIT ASSOCIATED WITH HIV INFECTION[6]

Depletion of CD4+ lymphocytes is a hallmark of HIV infection and AIDS, but this process is not fully understood. In addition, there is substantive evidence that demonstrable defects in T cell function are present before the decline in CD4+ lymphocytes occurs. HIV-infected individuals exhibit a selective impairment in their response to antigens to which they have been previously exposed through infection or vaccination. Such antigens are referred to as "recall" antigens. The immune response to recall antigens requires a subset of CD4+ lymphocytes bearing the cell surface markers CD29 and CD45 RO, which phenotypically define "memory" T cells. Recent studies suggest that this important population of memory T cells may be preferential targets for HIV infection both *in vitro* and *in vivo*. Exposure to a given antigen may stimulate an immune response in an HIV-infected individual, which may, paradoxically, result in depletion of the responding cells. Constriction of the T cell repertoire of specific antigenic recognition may thus occur prior to the depletion of total CD4+ lymphocytes. If this hypothesis proves to be correct, it would provide further support for early antiviral treatment of infected individuals. The impaired ability to respond to recall antigens also has important implications for therapeutic vaccination of seropositive individuals - a strategy designed to boost their antiviral immune response. HIV-infected individuals may be stratified to receive such immunization based on their ability to respond to other antigens.

REVERSING THE IMMUNE DEFICIT: CAN IT BE DONE?[7]

Numerous gaps remain in our knowledge of the immunopathogenesis of HIV infection and AIDS. The role of immune activation and the existence of possible cofactors for disease progression, such as coexisting viral (e.g., cytomegalovirus, Epstein-Barr virus) infection, require more complete description. The challenges faced in defining the pathogenic mechanisms of HIV infection highlight limitations in the current understanding of the human immune system.

Studies of T lymphocyte development in humans have been hampered by the inability to perform adaptive transfer studies, which have yielded essential insights into lymphoid differentiation in animal models. Recently, however, scientists have developed several models for the study of human lymphocyte development based on transfer of human lymphocytes, or their progenitors, into immunodeficient mice. These systems provide useful information regarding the identity of the human hematopoietic stem cell. Characterization of this stem cell is essential to many of the proposed strategies (such as autologous transplantation of genetically modified stem cells) for immunologic reconstitution of HIV-infected individuals. Gene therapy for HIV infection (so-called, "intracellular immunization") will require resolution of several outstanding concerns. Among them are: documentation that the pluripotent stem cell is not itself a target for HIV infection and the development of an efficient means to genetically modify

[6]This section is based on material presented by Gene Shearer.

[7]This section is based on material presented by Irving Weissman.

stem cells. Further investigation is needed to define the developmental potential of T lymphocytes in adult humans, as well as to understand the consequences of viral infection of important lymphoid organs, such as the thymus.

IMMUNE RESPONSE TO HIV[8]

The fluctuation in virus production at various stages of the disease process indicates that the immune system may contain, albeit temporarily, HIV replication. Studies of the immune response to HIV infection have shown that both humoral and cytotoxic responses are directed against viral antigenic determinants. Researchers have devoted substantial effort to defining the HIV antigens (and epitopes) that antibodies and cytotoxic T cells recognize. Yet, at present, knowledge is limited regarding the potentially protective components of the antiviral immune response. Identifying these protective determinants, as well as the agent of host immunity that may effectively respond to them, is an essential challenge for future HIV vaccine development efforts.

Scientists have focused considerable attention on the phenomenon of antibody-mediated neutralization of HIV-1 *in vitro*. The primary neutralization domain has been mapped to a region of the gp12O envelope glycoprotein, referred to as the V3 loop. A large proportion of the antibodies in naturally infected humans or experimentally immunized animals, which are able to neutralize HIV-1 infectivity *in vitro*, do so by binding to the V3 loop. The precise mechanism of neutralization is as yet unclear. Antibodies experimentally generated against the V3 loop are generally type-specific; that is, they are only able to neutralize the viral isolate from which the sequences used for immunization were derived. Sera from HIV-1 infected patients generally display more broadly reactive neutralizing abilities. The V3 loop designation refers to the third variable region of the gp12O molecule. It, by definition, displays considerable sequence variation among viral isolates. Because such variation may frustrate vaccine development efforts, immunogens that elicit broadly neutralizing antibodies are being investigated. Effective immunogens may be identified through the study of conserved elements within the V3 loop or through population studies to define the extent and localization of HIV-1 variants. Challenge studies conducted among chimpanzees have shown that monoclonal antibodies with strong reactivity to the V3 loop can prevent infection. These studies offer provisional support for the potential of vaccination with the V3 loop to protect against viral infection. Similar experiments conducted using human immune sera with *in vitro* neutralizing activity, however, have been unsuccessful.

Specific cell-mediated immune responses to HIV-1 are believed to be mediated by CD8+ T cells, which recognize peptide antigens bound to class I MHC (Major Histocompatibility Complex, also known as HLA in humans) molecules expressed on the surface of infected cells. Additional antiviral effector cells include natural killer (NK) cells and cells that carry out antibody-dependent cytotoxic activities. CD8+ T cells that react with HIV-1 antigens have been identified in a variety of tissues, including the peripheral blood, lymph nodes, skin, central nervous system, and lung. In certain instances, these cells may contribute to the pathologic consequences of HIV-1 infection in affected tissues. However, the balance between the protective or possibly pathogenic role of CD8+ antiviral T cells is not known.

Because human T cell populations are genetically heterogeneous and CD8+ T cells recognize antigens only in the context of HLA (MHC Class 1) molecules, the identification of viral epitopes that are recognized by CD8+ T cells depends on the host cells' MHC haplotype. Although current evidence

[8]This section is based on material presented by Barry Bloom, Norman Letvin, and Wayne Koff.

suggests that CD8+ T cells can inhibit HIV-1 replication *in vitro*, the *in vivo* contribution of these cells to containment of HIV-1 infection is not known. Experimental resolution of this essential question will likely rely heavily on studies performed in primate animal model systems.

As discussed earlier, HIV-1 exhibits considerable sequence variation among isolates derived from different individuals. Sequence variation is also seen among different viruses within an individual over the course of infection. It is unclear whether the antiviral immune response selects for (or against) variation, or whether genetic drift permits HIV-1 to escape from immune surveillance. In the chimpanzee model, sera derived late in infection can neutralize early but not late viral isolates, which provides evidence for a process of viral escape. Whether this phenomenon exists in infected humans is uncertain. At present, the evidence regarding potential escape of viral variants from cytotoxic T cell recognition is both preliminary and conflicting.

Results from a variety of animal model systems are relevant to the development of a vaccine to prevent HIV-1 infection. In the SIV system, vaccines based on whole, inactivated virus have successfully protected immunized animals from intravenous experimental viral challenge. Subunit vaccines, which consist of the envelope glycoproteins alone, have been unsuccessful to date. In the chimpanzee model of HIV infection, animals immunized with recombinant HIV-1 gp120 or gp160 have been protected from experimental challenge. Important caveats must, however, accompany the results cited above. These experiments were performed under ideal conditions, which may bear little resemblance to prevention of natural HIV infection. All viral challenges in these experiments were of relatively low dose and consisted of free (not cell-associated) virus. In addition, they were delivered intravenously (not mucosally), at the height of the immune response, and they consisted of the same viral isolate used for immunization. Further vaccine studies will need to be performed under circumstances relevant to natural human exposures. Also critical to these efforts is an understanding of the nature of the protective immune response in immunized animals.

NEW AVENUES FOR DEVELOPING POTENTIALLY EFFECTIVE THERAPY[9]

Efforts directed toward the development of effective antiretroviral drugs are an essential part of the medical and scientific response to the HIV epidemic. Recent history in this area highlights both challenges and opportunities for future drug development efforts. Clinical experience with zidovudine (AZT) suggests one challenge - namely, therapeutic efficacy may require more than *in vitro* antiretroviral activity. Drug toxicity and potential development of drug-resistant viral variants are critically important considerations in developing drugs to treat a chronic disease.

Viral isolates with decreased sensitivity to zidovudine have been identified in treated patients. Although the clinical implications and correspondence of drug resistance are uncertain, it is a troubling development. The genetic alterations in HIV-1 isolates with decreased sensitivity to zidovudine have been mapped to a range of specific sites in the reverse transcriptase gene. The precise mechanism of zidovudine resistance induced by these mutations is not yet known.

Drug resistance has also emerged in HIV-1 infected patients treated with ddI, ddC, as well as with the non-nucleoside TIBO reverse transcriptase inhibitors. In some instances (e.g., TIBO drugs), resistance develops shortly after initiation of therapy. The phenomenon of antiviral drug resistance has prompted the application of combination therapy with simultaneous or alternating agents. The rationale for this therapeutic approach stems from the recognition that distinct genetic alterations are responsible for the

[9]This section is based on material presented by David Barry and Edward Scolnick.

development of resistance to particular antiviral agents, the varying toxicities of specific agents and, in some cases, their different mechanisms of action. It is hoped that combination therapy will be less toxic, more effective, and more likely to inhibit the emergence of drug-resistant viral variants. More definitive answers regarding combination antiretroviral therapy await the completion of ongoing clinical trials.

To date, many of the agents that are active against HIV infection have been identified through random screening of previously synthesized compounds. Exploitation of these compounds, however, depends on existing technology and expertise that derive from prior basic research efforts. Basic research, therefore, provides essential support for applied studies. Future progress in drug development efforts, including realization of the promise of "rational drug design," will depend on continued advances in basic science research.

Institute of Medicine

Roundtable for the Development of Drugs
and Vaccines Against AIDS

Understanding HIV Pathogenesis: Implications for Drug and Vaccine Development

July 26, 1991

Lecture Room, National Academy of Sciences
2101 Constitution Avenue, N.W.
Washington, D.C.

REVISED AGENDA

8:00 am	Continental Breakfast
8:15 am	Welcome and Introduction — Sheldon Wolff, Physician-in-Chief, New England Medical Center and Cochair, Roundtable
8:30 am	**VIRAL PATHOGENESIS OF HIV INFECTION AND AIDS: CURRENT UNDERSTANDING AND UNRESOLVED QUESTIONS** *Moderator*: David Baltimore, President, The Rockefeller University
8:35 am	Understanding the Viral Pathogenesis of HIV Infection and AIDS — Robert Gallo, Chief, Laboratory of Tumor Cell Biology, National Cancer Institute
8:55 am	Accessory Genes of HIV — Ronald Desrosiers, Chair, Department of Microbiology, New England Regional Primate Research Center
9:10 am	Biological Variability of HIV — Irvin Chen, Professor of Microbiology, Immunology and Medicine, UCLA School of Medicine
9:25 am	Discussion
10:25 am	Break
10:40 am	**IMMUNOPATHOGENESIS OF HIV INFECTION AND AIDS: CURRENT UNDERSTANDING AND UNRESOLVED QUESTIONS** *Moderator*: Max Cooper, Investigator, Howard Hughes Medical Institute, University of Alabama, Birmingham
10:45 am	Toward an Understanding of the Immunopathogenesis of HIV Infection and AIDS — Anthony Fauci, Director, National Institute of Allergy and Infectious Diseases

11:05 am — Immune Deficit Associated with HIV Infection
— Gene Shearer, Chief, Section on Cell-mediated Immunity, Experimental Immunology Branch, National Cancer Institute

11:20 am — Reversing the Immune Deficit: Can It Be Done?
— Irving Weissman, Professor, Department of Pathology, Stanford University School of Medicine

11:35 pm — Discussion

12:35 pm — Lunch

1:30 pm — **IMMUNE RESPONSE TO HIV**

Moderator: Barry Bloom, Professor and Chairman, Department of Microbiology and Immunology, Albert Einstein College of Medicine

1:35 pm — Viral Targets for Cellular and Humoral Immune Response
— Norman Letvin, Chair, Division of Immunology, New England Regional Primate Research Center

2:10 pm — Defining a Protective Immune Response
— Wayne Koff, Chief, Vaccine Research and Development Branch, Division of AIDS, National Institute of Allergy and Infectious Diseases

2:25 pm — Discussion

3:30 pm — Break

3:45 pm — **NEW AVENUES FOR DEVELOPING POTENTIALLY EFFECTIVE THERAPY**

Moderator: David Barry, Vice President of Research, The Wellcome Research Laboratories, Burroughs Wellcome Co.

3:50 pm — Identifying Novel Targets for Antiviral Intervention
— Edward Scolnick, President, Merck Sharp & Dohme Research Laboratories

4:05 pm — Discussion

4:45 pm — Concluding Remarks
— Sheldon Wolff, Physician-in-Chief, New England Medical Center and Cochair, Roundtable

5:00 pm — Adjourn